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REMARKS

I. Status of the Application and Claims

With entry of this Amendment, claims 1 and 3-40 are pending in the application. Office action, page 4. Claims 1-6, 8-20, 30, and 31 stand rejected. Claims 7, 21-29, and 32-40 have been withdrawn from consideration.

With respect to claim 7, however, in the prior Restriction Requirement the Office included claim 7 in elected Group I. See Paper No. 7, page 2. Given the subject matter of the claim, Applicants submit that the indication in the pending Office action that claim 7 has been withdrawn is in error. Accordingly, Applicants respectfully request that the Office clarify the status of claim 7.

Applicants have canceled claim 2 without prejudice or disclaimer of the subject matter recited therein.

To facilitate prosecution, and without prejudice or disclaimer, Applicants amend claim 6 to recite at least 35 consecutive nucleotides. The specification supports this amendment of claim 6 at paragraph 246.

Applicants have amended claim 9. In view of this amendment, Applicants respectfully request the Office to consider the patentability of claim 9 on the merits.

Applicants note that there were two claims each identified as claim 16 in the application as filed, and no claim identified as claim 15. To correct this typographical error, Applicants have amended the first instance of claim 16 to recite claim 15, thus clarifying the numbering of these two claims.

Applicants have enclosed a substitute Sequence Listing, which among other things, adds SEQ ID NOS. 47 and 48 for the ABCC11 and ABCC5 amino acid sequences set forth in Figure 1.

The Office objects to the specification because the description of Figure 1 does not provide the SEQ ID NOS. of the sequences in the Figure. Office action, page 4. Applicants have amended Figure 1's description, at paragraph 110, to indicate these SEQ ID NOS. Applicants request withdrawal of the objection.

Figure 1 is also objected to because it also does not provide the SEQ ID NOS. of the sequences in the figure. *Id.* Applicants file herewith a replacement Figure 1, which contains the SEQ ID NOS. As both of these objections have been rendered moot, Applicants request that the Office withdraw them.

II. Rejection Under 35 U.S.C. §§ 101/112, First Paragraph

Claims 1-6, 8-20, 30, and 31 stand rejected as allegedly lacking a patentable utility. Office action, page 6. Applicants traverse the rejection.

The invention of claims 1-6, 8-20, 30, and 31 does have a specific, substantial, and credible utility as evidenced by the enclosed article by T. K. Bera *et al.*, MRP9, An Unusual Truncated Member of the ABC Transporter Superfamily, Is Highly Expressed in Breast Cancer, PNAS-USA 99:6997-7002 (2002) ("Bera"). According to Bera, the ABCC12 gene, which is also known as MRP9, produces two major mRNA transcripts, one 4.5 kb and the other 1.3 kb in length. *Id.*, page 6997. Breast cancer cells express the 4.5 kb transcript at high levels while normal tissues express that transcript in very low levels or not at all. *Id.*, pages 6997, 7000, and 7001. As the authors in Bera note, the ABCC12 gene can be used to develop immunotherapy treatments for breast cancer. *Id.*, page 7002. In addition, the ABCC12 gene could be used to detect breast cancer in mRNA samples from breast tissue samples.

The specification also considers the ABCC12 gene's application to cancer. Multiple drug resistance phenotypes in tumor cells have been associated with the multi-drug resistance protein, which has an ABC transporter structure. Specification, paragraph 005. Several ABC transporter family protein members are in the multidrug resistance-like (MRP) subgroup, including ABCC 5. *Id.*, paragraph 006. ABCC5 is highly related to ABCC12. *Id.*, Figure 4. Because multi-drug resistance is associated with cancer and ABCC12 is highly related to ABCC5, a known MRP, it follows that ABCC12 may also be involved in cancer. *Id.*, paragraph 008 ("Since structurally related ABC proteins often transport similar substrates across the membranes, it would be reasonable to suggest that the ABCC12 proteins could share functional similarities with ABCC4 and/or ABCC5 genes . . . .") Bera confirms that ABCC12 gene is in the MRP subgroup by referring to the ABCC12 gene as MRP9. Thus, given itsteaching, the specification contemplates an application of the ABCC12 gene to the treatment and/or diagnosis of various forms of cancer.

In view of these remarks, Applicants submit that there is a specific, substantial, and credible utility for the claimed invention, and therefore the claims are also enabled. Accordingly, Applicants request the Office to withdraw the rejection for lack of utility under section 101 and the related rejection for nonenablement under section 112, first paragraph.

III. Rejections Under 35 U.S.C. § 112, First Paragraph

The Office rejects claims 3-5, 8, and 10 under 35 U.S.C. § 112, first paragraph, for allegedly failing to comply with the written description requirement. Office action, page 11. According to the Office, these claims encompass variant forms of the claimed nucleic acids for which the specification allegedly lacks written description. *Id.,* page 12. Specifically, the Office believes that claims 3-5, 8, and 10 encompass alternate splice variants, insertions, and mutations, but only one such variant, SEQ ID NO. 2, is disclosed in the specification. *Id.,* pages 12 and 13. Applicants traverse this rejection.

Applicants submit that the Office's own guidelines for examining claims for compliance with the written description requirement demonstrate that the specification supports claims 3-5, 8, and 10. Synopsis of Application of Written Description Guidelines, available at http://www.uspto.gov/web/menu/written.pdf. Example 9 (Synopsis, page 35), involves a hypothetical claim to an isolated nucleic acid that specifically hybridizes under highly stringent conditions to the complement of the sequence set forth in SEQ ID NO: 1, wherein said nucleic acid encodes a protein that binds to a dopamine receptor and stimulates adenylate cyclase activity. In this Example, the Office reasons that the written description requirement is satisfied because the hypothetical specification indicates that a main feature of the claimed invention is the isolated nucleic acid that hybridizes to SEQ ID NO: 1 under highly stringent conditions and encodes a protein with a specific function. *See Id.* "The art indicates that hybridization techniques using a known DNA as a probe under highly stringent conditions were conventional in the art at the time of filing." *Id.* The Synopsis continues its analysis, noting that "a person of skill in the art would not expect

substantial variation among species encompassed within the scope of the claims because the highly stringent hybridization conditions set forth in the claim yield structurally similar DNAs." *Id.* The Office concludes that "highly stringent hybridization conditions in combination with the coding function of DNA and the level of skill and knowledge in the art are adequate to determine that applicant was in possession of the claimed invention." *Id.*

In the instant case, claim 5, for example, recites hybridization under high stringency conditions. The specification clearly describes the concept of high stringency. Specification, paragraph 154. The common relationship that all nucleic acids of claim 5 share is the ability to hybridize with a nucleic acid comprising any one of SEQ ID NOS: 1-32 or the complement of such sequences. As described above, hybridization techniques using a known DNA as a probe under highly stringent conditions were conventional in the art at the time of filing. These highly stringent hybridization conditions in combination with the coding function of DNA (encoding a transporter protein) and the level of skill and knowledge in the art support the conclusion, consistent with the Synopsis example, that Applicants' specification provides written description support for the claimed invention.

In sum, claims 3-5, 8, and 10 do have written description support in the specification. Applicants request that the Office reconsider and withdraw this rejection.

IV. Rejection Under 35 U.S.C. § 112, Second Paragraph

The Office rejects claims 9-16 as allegedly indefinite because claims 9, 11-13, and 15 recite "a nucleotide primer as in any one of claims 6-8" and claim 8 is allegedly not drawn to a primer. Office action, page 14. Applicants traverse the rejection.

Solely to advance the prosecution of this case, however, Applicants have amended claim 9 to remove the reference to claim 8 in the body of the claim. IN view of this amendment, Applicants submit that the rejection is moot and they request that the Office reconsider and withdraw it.

V. Rejections Under 35 U.S.C. § 102

A. U.S. Patent No. 5,721,098 to Pinkel *et al.*

Claims 1-5 stand rejected as allegedly anticipated under 35 U.S.C. § 102(b) by U.S. Patent No. 5,721,098 to Pinkel *et al.* ("Pinkel"). According to the Office, Pinkel teaches isolated chromosome 16 and the specification discloses that SEQ ID NO: 1 was mapped to this chromosome. Office action, page 15. The Office concludes that Pinkel inherently teaches nucleic acids comprising 8 consecutive nucleotides and a nucleic acid with 80 % identity to SEQ ID NO: 1. *Id.* Pinkel also allegedly discloses the hybridization of 600 PE DNA to chromosome 16. *Id.* Applicants traverse the rejection, which is moot as to claim 2 because that claim has been canceled.

SEQ ID NO: 1 is a cDNA sequence. *See* specification at paragraph 224. As is known in the art, cDNA sequences are different from chromosomal sequences in that cDNA sequences do not contain introns. Thus, a gene for ABCC12 located on chromosome 16 would contain contains both exon and intron sequences. Accordingly, that sequence would not anticipate the isolated nucleic acids of claims 1 and 3-5. In view of these remarks. Applicants request that the Office withdraw this rejection.

B. U.S. Patent No. 5,994, 130 to Shyjan

The Office also rejects claims 2, 5, 6, and 12 as allegedly anticipated under 35 U.S.C. § 102(b) by U.S. Patent No. 5,994,130 to Shyjan ("Shyjan"). Office action, page

15. The Office contends that Shyjan teaches a nucleic acid sequence containing 19 consecutive nucleotides identical to 19 consecutive nucleotides in SEQ ID NO: 1. *Id.*, pages 15 and 16. As claim 2 has been canceled, Applicants respond with respect to claims 5, 6, and 12.

Regarding claim 5, Shyjan's SEQ ID NO: 1 is 4,781 base pairs long. *See* Shyjan Sequence Listing. Even if the Office's reading of Shyjan is correct, and Shyjan's sequence shares 19 consecutive nucleotides with SEQ ID NO: 1, only 0.3% of Shyjan's sequence is homologous to the instant SEQ ID NO: 1. The skilled artisan would readily recognize that a DNA molecule with such a low level of homology to SEQ ID NO: 1 would not hybridize under high stringency conditions.

Applicants have amended claim 6 to recite at least 35 consecutive nucleotides. Thus, Shyjan, which the Office contends discloses 19 consecutive nucleotides identical to SEQ ID NO: 1, does not anticipate claim 6. As claim 12 is dependent on claim 6, Shyjan also does not anticipate that claim.

In view of these remarks and the above amendments, Applicants request withdrawal of this rejection.

C. <u>Application U.S. 2003/0032021 to Curtis</u>

The Office rejects claims 2, 5, 6, and 12 as allegedly anticipated under 35 U.S.C. § 102(e) by U.S. Application No. 2003/0032021 to Curtis ("Curtis"). Office action, page 16. The Office asserts that Curtis teaches a nucleic acid sequence that contains 31 consecutive nucleotides that are identical to 31 consecutive nucleotides in SEQ ID NO: 1. *Id.* As claim 2 has been canceled, Applicants respond with respect to claims 5, 6, and 12.

The same arguments applied above with respect to Shyjan also apply to the instant rejection. Curtis' SEQ ID NO: 3 is 463 base pairs long. Even if the Office's reading of Curtis is correct, and Curtis's sequence shares 31 consecutive nucleotides with the instant SEQ ID NO: 1, only 7% of Curtis's sequence is homologous to the instant SEQ ID NO: 1. The skilled artisan would readily recognize that a DNA molecule with such a low level of homology to SEQ ID NO: 1 would not hybridize under high stringency conditions. Accordingly, Applicants submit that Curtis does not anticipate claim 5.

Regarding claim 6, Applicants have amended this claim to recite at least 35 consecutive nucleotides. Thus, Curtis, which allegedly discloses 31 consecutive nucleotides identical to SEQ ID NO: 1, does anticipate claim 6. As claim 12 is dependent on claim 6, Curtis also does not anticipate claim 12.

For these reasons, Applicants request that the Office reconsider and withdraw this rejection.

D. Peng *et al.*

Claim 10 stands rejected as allegedly anticipated under 35 U.S.C. § 102(b) by Peng *et al.*, Multiple PCR Analyses on Trace Amounts of DNA extracted from Freshand Parafin Wax Embedded Tissues after Random Hexamer Primer PCR Amplification., *J. Clin. Pathol.*, 47:605-08 (1994) ("Peng"). Office action, page 17. Applicants traverse.

Claim 10 recites "[a] kit for amplifying the nucleic acid according to claim 1 . . . ." Peng teaches the use of random primers in PCR reactions. The primers are not specifically designed to hybridize to the nucleic acid recited by claim 1. Peng, therefore, does not expressly anticipate claim 10.

Nor has the Office provided any evidence or reasoning showing that the random primers of Peng **necessarily** amplify the nucleic acid according to claim 1. Accordingly, Peng does not inherently anticipate claim 10.

For these reasons, Applicants request reconsideration and withdrawal of this rejection.

Conclusion

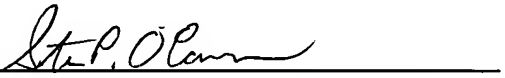
In view of the foregoing amendments and remarks, Applicants respectfully request reconsideration and reexamination of this application and the timely allowance of the claims under consideration.

Please grant any extensions of time required to enter this response and charge any additional required fees to our deposit account 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Dated: April 20, 2004

By: 
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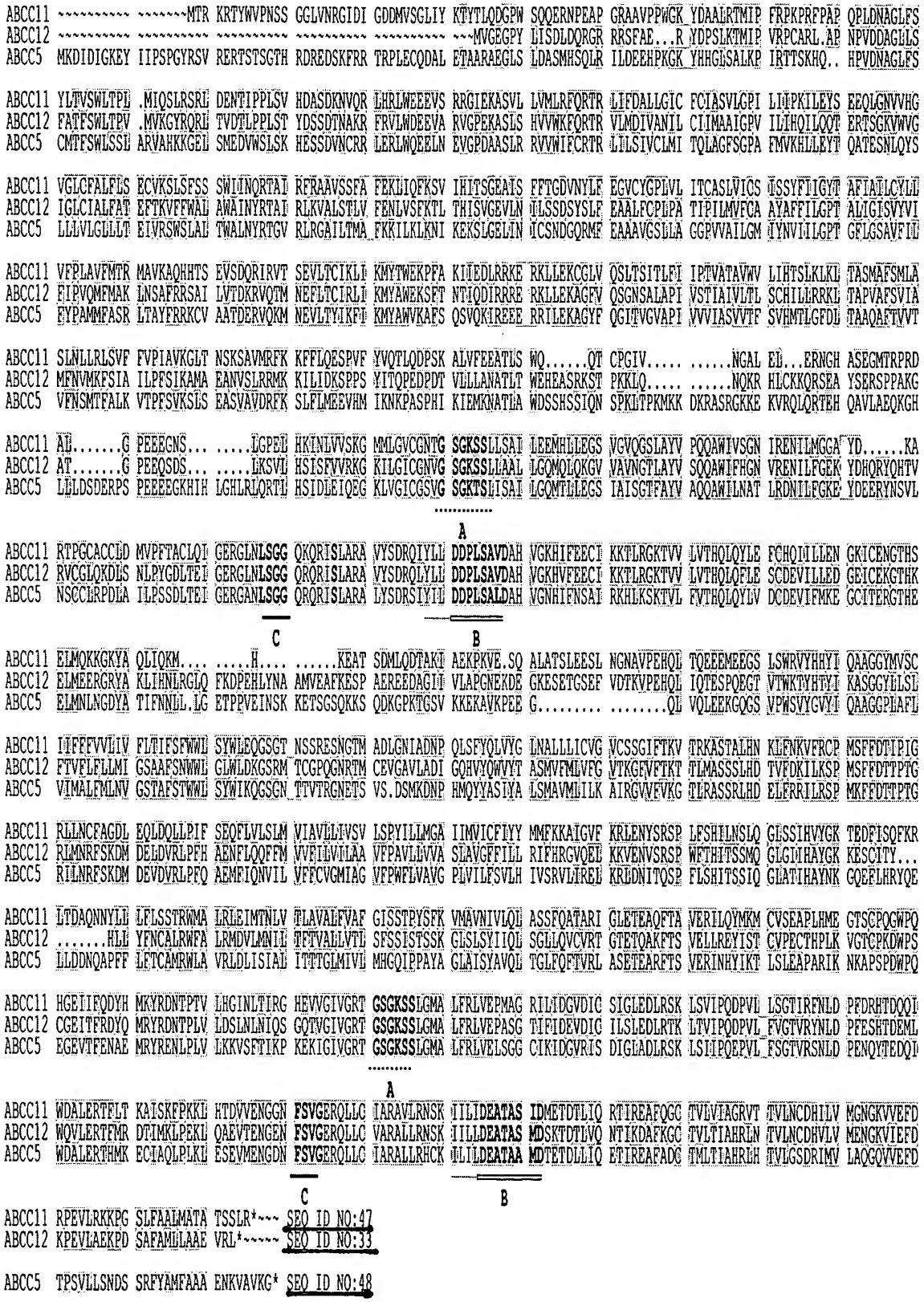

FIG. 1

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ABCC11 ~~~~~~~~~~ ~~~~~~~MTR KRTYWVPNSS GGLVNRGIDI GDDMVSGLIY KTYTLQDGPW SQQERNPEAP GRAAVPPWGK YDAAIRTMIP FRPKPRFPAP QPLDNAGLFS
ABCC12 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~MVGEGPY LISDLDQRGR RRSFAE...R YDPSLKTMIP VRPCARL.AP NPVDDAGLLS
ABCC5  MKDIDIGKEY IIPSPGYRSV RERTSTSGTH RDREDSKFRR TRPLECQDAL ETAARAEGLS LDASMHSQLR ILDEEHPKGK YHHGLSALKP IRTTSKHQ.. HPVDNAGLFS

ABCC11 YLTVSWLTPL .MIQSLRSRL DENTIPPLSV HDASDKNVQR LHRLWEEEVS RRGIEKASVL LVMLRFQRTR LIFDALLGIC FCIASVLGPI LIIPKILEYS EEQLGNVVHG
ABCC12 FATFSWLTPV .MVKGYRQRL TVDILPPLST YDSSDTNAKR FRVLWDEEVA RVGPEKASLS HVVWKFQRTR VLMDIVANIL CIIMAAIGPV ILIHQILQQT ERTSGKVWVG
ABCC5  CMTFSWLSSL ARVAHKKGEL SMEDVWSLSK HESSDVNCRR LERLWQEELN EVGPDAASLR RVVWIFCRTR LILSIVCLMI TQLAGFSGPA FMVKHLLEYT QATESNLQYS

ABCC11 VGLCFALFLS ECVKSLSFSS SWIINQRTAI RFRAAVSSFA FEKLIQFKSV IHITSGEAIS FFTGDVNYLF EGVCYGPLVL ITCASLVICS ISSYFIIGYT AFIAILCYLL
ABCC12 IGLCIALFAT EFTKVFFWAL AWAINYRTAI RLKVALSTLV FENLVSFKTL THISVGEVLN ILSSDSYSLF EAALFCPLPA TIPILMVFCA AYAFFILGPT ALIGISVYVI
ABCC5  LLLVLGLLLT EIVRSWSLAL TWALNYRTGV RLRGAILTMA FKKILKLKNI KEKSLGELIN ICSNDGQRMF EAAAVGSLLA GGPVVAILGM IYNVIILGPT GFLGSAVFIL

ABCC11 VFPLAVFMTR MAVKAQHHTS EVSDQRIRVT SEVLTCIKLI KMYTWEKPFA KIIEDLRRKE RKLLEKCGLV QSLTSITLFI IPTVATAVWV LIHTSLKLKL TASMAFSMLA
ABCC12 FIPVQMFMAK LNSAFRRSAI LVTDKRVQTM NEFLTCIRLI KMYAWEKSFT NTIQDIRRRE RKLLEKAGFV QSGNSALAPI VSTIAIVLTL SCHILLRRKL TAPVAFSVIA
ABCC5  FYPAMMFASR LTAYFRRKCV AATDERVQKM NEVLTYIKFI KMYAWVKAFS QSVQKIREEE RRILEKAGYF QGITVGVAPI VVVIASVVTF SVHMTLGFDL TAAQAFTVVT

ABCC11 SLNLLRLSVF FVPIAVKGLT NSKSAVMRFK KFFLQESPVF YVQTLQDPSK ALVFEEATLS WQ......QT CPGIV..... .....NGAL EL...ERNGH ASEGMTRPRD
ABCC12 MFNVMKFSIA ILPFSIKAMA EANVSLRRMK KILIDKSPPS YITQPEDPDT VLLLANATLT WEHEASRKST PKKLQ..... .....NQKR HLCKKQRSEA YSERSPPAKG
ABCC5  VFNSMTFALK VTPFSVKSLS EASVAVDRFK SLFLMEEVHM IKNKPASPHI KIEMKNATLA WDSSHSSIQN SPKLTPKMKK DKRASRGKKE KVRQLQRTEH QAVLAEQKGH

ABCC11 AL.......G PEEEGNS... .....LGPEL HKINLVVSKG MMLGVCGNTG SGKSSLLSAI LEEMHLLEGS VGVQGSLAYV PQQAWIVSGN IRENILMGGA YD......KA
ABCC12 AT.......G PEEQSDS... .....LKSVL HSISFVVRKG KILGICGNVG SGKSSLLAAL LGQMQLQKGV VAVNGTLAYV SQQAWIFHGN VRENILFGEK YDHQRYQHTV
ABCC5  LLLDSDERPS PEEEEGKHIH LGHLRLQRTL HSIDLEIQEG KLVGICGSVG SGKTSLISAI LGQMTLLEGS IAISGTFAYV AQQAWILNAT LRDNILFGKE YDEERYNSVL

                                                                  ..........
                                                                  A
ABCC11 RTPGCACCLD MVPFTACLQI GERGLNLSGG QKQRISLARA VYSDRQIYLL DDPLSAVDAH VGKHIFEECI KKTLRGKTVV LVTHQLQYLE FCHQIILLEN GKICENGTHS
ABCC12 RVCGLQKDLS NLPYGDLTEI GERGLNLSGG QRQRISLARA VYSDRQLYLL DDPLSAVDAH VGKHVFEECI KKTLRGKTVV LVTHQLQFLE SCDEVILLED GEICEKGTHK
ABCC5  NSCCLRPDLA ILPSSDLTEI GERGANLSGG QRQRISLARA LYSDRSIYIL DDPLSALDAH VGNHIFNSAI RKHLKSKTVL FVTHQLQYLV DCDEVIFMKE GCITERGTHE

                                  ----------
                                  C          B
ABCC11 ELMQKKGKYA QLIQKM.... .....H.... .....KEAT SDMLQDTAKI AEKPKVE.SQ ALATSLEESL NGNAVPEHQL TQEEEMEEGS LSWRVYHHYI QAAGGYMVSC
ABCC12 ELMEERGRYA KLIHNLRGLQ FKDPEHLYNA AMVEAFKESP AEREEDAGIL VLAPGNEKDE GKESETGSEF VDTKVPEHQL IQTESPQEGT VTWKTYHTYI KASGGYLLSL
ABCC5  ELMNLNGDYA TIFNNLL.LG ETPPVEINSK KETSGSQKKS QDKGPKTGSV KKEKAVKPEE G..... .....QL VQLEEKGQGS VPWSVYGVYI QAAGGPLAFL
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FIG. 1A

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ABCC11 IIFFFVVLIV FLTIFSFWWL SYWLEQGSGT NSSRESNGTM ADLGNIADNP QLSFYQLVYG LNALLLICVG VCSSGIFTKV TRKASTALHN KLFNKVFRCP MSFFDTIPIG
ABCC12 FTVFLFLLMI GSAAFSNWWL GLWLDKGSRM TCGPQGNRTM CEVGAVLADI GQHVYQWVYT ASMVFMLVFG VTKGFVFTKT TLMASSSLHD TVFDKILKSP MSFFDTTPTG
ABCC5  VIMALFMLNV GSTAFSTWWL SYWIKQGSGN TTVTRGNETS VS.DSMKDNP HMQYYASIYA LSMAVMLILK AIRGVVFVKG TLRASSRLHD ELFRRILRSP MKFFDTTPTG

ABCC11 RLLNCFAGDL EQLDQLLPIF SEQFLVLSLM VIAVLLIVSV LSPYILLMGA IIMVICFIYY MMFKKAIGVF KRLENYSRSP LFSHILNSLQ GLSSIHVYGK TEDFISQFKR
ABCC12 RLMNRFSKDM DELDVRLPFH AENFLQQFFM VVFILVILAA VFPAVLLVVA SLAVGFFILL RIFHRGVQEL KKVENVSRSP WFTHITSSMQ GLGIIHAYGK KESCITY...
ABCC5  RILNRFSKDM DEVDVRLPFQ AEMFIQNVIL VFFCVGMIAG VFPWFLVAVG PLVILFSVLH IVSRVLIREL KRLDNITQSP FLSHITSSIQ GLATIHAYNK GQEFLHRYQE

ABCC11 LTDAQNNYLL LFLSSTRWMA LRLEIMTNLV TLAVALFVAF GISSTPYSFK VMAVNIVLQL ASSFQATARI GLETEAQFTA VERILQYMKM CVSEAPLHME GTSCPQGWPQ
ABCC12 .......HLL YFNCALRWFA LRMDVLMNIL TFTVALLVTL SFSSISTSSK GLSLSYIIQL SGLLQVCVRT GTETQAKFTS VELLREYIST CVPECTHPLK VGTCPKDWPS
ABCC5  LLDDNQAPFF LFTCAMRWLA VRLDLISIAL ITTTGLMIVL MHGQIPPAYA GLAISYAVQL TGLFQFTVRL ASETEARFTS VERINHYIKT LSLEAPARIK NKAPSPDWPQ

ABCC11 HGEIIFQDYH MKYRDNTPTV LHGINLTIRG HEVVGIVGRT GSGKSSLGMA LFRLVEPMAG RILIDGVDIC SIGLEDLRSK LSVIPQDPVL LSGTIRFNLD PFDRHTDQQI
ABCC12 CGEITFRDYQ MRYRDNTPLV LDSLNLNIQS GQTVGIVGRT GSGKSSLGMA LFRLVEPASG TIFIDEVDIC ILSLEDLRTK LTVIPQDPVL FVGTVRYNLD PFESHTDEML
ABCC5  EGEVTFENAE MRYRENLPLV LKKVSFTIKP KEKIGIVGRT GSGKSSLGMA LFRLVELSGG CIKIDGVRIS DIGLADLRSK LSIIPQEPVL FSGTVRSNLD PENQYTEDQI

ABCC11 WDALERTFLT KAISKFPKKL HTDVVENGGN FSVGERQLLC IARAVLRNSK IILIDEATAS IDMETDTLIQ RTIREAFQGC TVLVIAGRVT TVLNCDHILV MGNGKVVEFD
ABCC12 WQVLERTFMR DTIMKLPEKL QAEVTENGEN FSVGERQLLC VARALLRNSK IILLDEATAS MDSKTDTLVQ NTIKDAFKGC TVLTIAHRLN TVLNCDHVLV MENGKVIEFD
ABCC5  WDALERTHMK ECIAQLPLKL ESEVMENGDN FSVGERQLLC IARALLRHCK ILILDEATAA MDTETDLLIQ ETIREAFADC TMLTIAHRLH TVLGSDRIMV LAQGQVVEFD

ABCC11 RPEVLRKKPG SLFAALMATA TSSLR*~~~ SEQ ID NO:47
ABCC12 KPEVLAEKPD SAFAMLLAAE VRL*~~~~~ SEQ ID NO:33

ABCC5  TPSVLLSNDS SRFYAMFAAA ENKVAVKG* SEQ ID NO:48
```

A

B

C

FIG. 1B

MRP9, an unusual truncated member of the ABC transporter superfamily, is highly expressed in breast cancer

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Contributed by Ira Pastan, April 1, 2002

Combining a computer-based screening strategy and functional genomics, we previously identified MRP9 (ABCC12), a member of the ATP-binding cassette (ABC) superfamily. We now show that the gene has two major transcripts of 4.5 and 1.3 kb. In breast cancer, normal breast, and testis, the *MRP9* gene transcript is 4.5 kb in size and encodes a 100-kDa protein. The protein is predicted to have 8 instead of 12 membrane-spanning regions. When compared with closely related ABC family members, it lacks transmembrane domains 3, 4, 11, and 12 and the second nucleotide-binding domain. In other tissues including brain, skeletal muscle, and ovary, the transcript size is 1.3 kb. This smaller transcript encodes a nucleotide-binding protein of ~25 kDa in size. An *in situ* hybridization study shows that the 4.5-kb transcript is expressed in the epithelial cells of breast cancer. An antipeptide antibody designed to react with the amino terminus of the protein detects a 100-kDa protein in testis and the membrane fraction of a breast cancer cell line. Because the 4.5-kb RNA is highly expressed in breast cancer and not expressed at detectable levels in essential normal tissues, MRP9 could be a useful target for the immunotherapy of breast cancer. Because of the unusual topology of the two variants of *MRP9*, we speculate that they may have a different function from other family members.

Completion of the human genome project and advances in bioinformatics have enabled researchers to identify and analyze new genes that could be used as targets for cancer therapy or could be involved in the multistep process of cancer. Many different methods now are used to identify tissue- or cancer-specific genes. Over the past several years our laboratory has identified genes expressed in prostate cancer and normal prostate by using the expressed sequence tag (EST) database (1, 2). In this approach a computer-based screening strategy is used to generate clusters of ESTs that are expressed specifically in normal prostate and/or prostate cancer but not in essential normal tissues (3). Several new prostate-specific genes have been identified by this approach (4–8). With the publication of the draft sequence of the human genome we have been able, in most cases, to identify the gene encoding each EST cluster and determine whether the protein has the characteristics of a membrane protein. Our laboratory is focused on the development of immunotoxin for the therapy of cancer (9). For this therapy and other antibody-based therapies to be effective, it is essential that the target antigen be a membrane-associated protein located on the cell surface. Using this approach, we recently reported the identification of *MRP8* (ABCC11), a member of the ATP-binding cassette (ABC) transporter superfamily, which is highly expressed in breast cancer (10) and of MRP9 (ABCC12). In this report, we have analyzed the RNA transcripts and protein produced by MRP9. The *MRP9* gene is unusual because it encodes two transcripts of different sizes. The larger 4.5-kb RNA is found in breast cancer, normal breast, and testis and encodes an MRP-like protein that lacks transmembrane domains 3, 4, 11, and 12 and the second nucleotide-binding domain. The smaller 1.3-kb RNA is detected in brain, skeletal muscle, and ovary and seems to encode the second nucleotide-binding domain.

Materials and Methods

EST Database Mining and Computer Analysis. The methods used for database analysis of ESTs and the alignment of the individual EST with the genomic sequence was described earlier (3, 10).

RNA Dot Blots and Northern Blot Hybridization. RNA hybridization was performed on multiple-tissue Northern blots (CLONTECH) and a human multiple-tissue expression array (CLONTECH, catalog no. 7775-1) containing mRNA from 76 human tissues in separate dots as described earlier (10). The 400-bp PCR fragment generated by primers T385 and T386 was used as a 3′-specific probe. The 600-bp (nucleotides 1–600) DNA fragment was used as a 5′-specific probe. The sequences of the primers used in this study are listed in Table 1.

Reverse Transcription (RT)-PCR Analysis on a Gene-Expression Panel. A rapid-scan gene-expression panel containing PCR-ready first-strand cDNA from 24 different tissues (OriGene, Rockville, MD, catalog no. HSCA-101) was used as a template for PCR with a primer pair (T385 and T386) that should give a 400-bp fragment. For expression analysis of *MRP8* in normal breast and breast cancer, we used a human breast cancer rapid-scan panel (OriGene catalog no. TSCE-101) that contains PCR-ready first-strand cDNA from 12 normal and 12 breast cancer tissues. PCR composition and conditions used were according to the supplier's instructions.

Cloning of the Full-Length cDNA. Rapid amplification of cDNA ends (RACE) was performed on Marathon Ready brain and testis cDNA (CLONTECH). Gene-specific primers T385 and T386 were used for the 3′ and 5′ RACE, respectively. The PCR product was gel-purified and cloned into the pCR2.1 TOPO vector (Invitrogen). The longest clones were identified by restriction digestion and sequenced by using a rhodamine terminator sequencing kit (Perkin–Elmer Applied Systems, Warrington, U.K.).

Antibody Production and Purification of IgG from Antisera. A peptide of 14 aa (amino acids 15–28) was synthesized, conjugated with

Abbreviations: EST, expressed sequence tag; RT, reverse transcription; ABC, ATP-binding cassette; RACE, rapid amplification of cDNA ends.

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Table 1. Sequences of primers used in this study

Primer name	Primer sequence
T385	GCA TTC TCA GCT TGG AAG ACC TCA
T386	CTT CTC TGC AAG GAC TTC AGG CTT
T396	AGC ACC AGC TCA TCC AGA CTG AAT
T399	TGA GTG CAT TCA GGA ACA CAG GTC
T412	CCA CAG AGG AGT CCA GGA GCT CAA
T413	TGG CAA GAA GGA GAG CTG CAT CAC
T414	TGT GGC CTT GTT GGT GAC CCT GAG
T415	GAC GCA AGC CAA ATT CAC CTC CGT
T418	GGA GAG CTG CAT CAC CTA TCA CCT
T419	GGA GAG CTG CAT CAC CTA GTT TAA

keyhole limpet hemocyanin, and injected into rabbits with complete Freund' s adjuvant for the first immunization and incomplete Freund's adjuvant for subsequent immunizations. Sera were collected after the fourth and fifth immunizations and analyzed by ELISA against the synthesized peptide. Total IgG was purified with immobilized protein A (Pierce) following the supplier's instructions.

Western Blot Analysis. Approximately 40 μg of protein extract from different tissues (Protein Medley, CLONTECH) or the 100,000 $\times$ *g* pellet of a homogenate of the CRL1500 breast cancer cell line were separated on a 10% Tris-glycine gel (Bio-Rad) and transferred to a 0.2-μm Immun-Blot polyvinylidene difluoride membrane (Bio-Rad) in transfer buffer [25 mM Tris/192 mM glycine/0% (vol/vol) methanol, pH 8.3] at 4°C for 2 h at 50 V. Filters were probed with 10 μg/ml protein A-purified anti-*MRP9* antiserum or preimmune serum, and their respective signals were detected by using a chemiluminescence Western blotting kit according to instructions from Roche Molecular Biochemicals as described (11).

***In Situ* Hybridization.** Pretreatment of the tissue sections for *in situ* hybridization was performed as described (8). Biotinylated cDNA probes were prepared by using a 600-bp fragment from the 5′ end of *MRP9* and full-length U6 (a small nuclear RNA known to be expressed in almost all cells) cloned in pBluescript II SK(+) plasmid. Biotinylated pBluescript II SK(+) with a CD22 insert was used as a negative control. Probe labeling, hybridization, and washing conditions were similar to those described previously (8). Microscopic evaluation (bright-field) was performed by using a Nikon Eclipse 800 microscope (12).

***In Vitro* Transcription-Coupled Translation.** The *in vitro* translation of the 4.5-kb variant of MRP9 cDNA from testis was examined in an *in vitro* transcription-coupled translation system (TNT, Promega). [^{35}S]Met (ICN) was incorporated in the reaction for visualization of translated products. The reaction mixture was analyzed under reducing conditions on a polyacrylamide gel (7.5% Tris/glycine, Bio-Rad) together with a prestained marker (Bio-Rad) and autoradiographed.

Results

Identification of *MRP9* (ABCC12). We recently reported (8) that *MRP8*, a member of the ABC transporter superfamily, is located in a genomic region of over 80.4 kb on chromosome 16q12.1. Using the GENESCAN gene prediction program (13) we identified an adjacent gene with homology to *MRP8* and named it *MRP9* (10). When the predicted cDNA of *MRP9* was analyzed to identify SAGE tags by using the SAGE map database (www.ncbi.nlm.nih.gov/SAGE/), the sequence matches up with five tags; four are from breast cancer, and one is from pancreatic cancer, indicating that *MRP9* may be expressed commonly in breast cancer (data not shown).

Experimental Analysis of *MRP9* Transcripts. To determine the tissue specificity of *MRP9* expression, we performed a multitissue dot blot analysis by using a PCR-generated DNA fragment from the 3′ end of the predicted *MRP9* gene (Fig. 1*B*). PCR primers T385 and T386 were designed from the predicted cDNA sequence, and a PCR product of the expected size was amplified, cloned, and sequenced from testis cDNA. The DNA fragment was labeled with ^{32}P by random priming and used for dot blot hybridization. As shown in Fig. 2*A*, among the 76 different samples of normal and fetal tissues examined *MRP9* is detected in different parts of the brain (1A–1G, A2, D2, F2, and B3), testis (F8), and pancreas (B9).

To confirm the dot blot result we used a more sensitive PCR-based analysis to validate tissue-specific expression of *MRP9*. In this analysis we used a panel of cDNAs isolated from 24 different normal tissues and performed PCRs with a primer pair (T385 and T386) located at the 3′ end of the MRP9 (Fig. 1). (The same primer pair was used to generate a probe for the dot blot analysis designed from the 3′-DNA sequence of *MRP9*.) As shown in Fig. 2*C*, a specific band of 400 bp is detected in normal brain (lane 1), testis (lane 11), ovary (lane 17), and skeletal muscle (lane 9). There also is a weak but detectable signal in pancreas (lane 16).

Analysis of *MRP9* Transcript in Different Tissues. To determine the transcript size of the *MRP9* mRNA, we performed an analysis of a Northern blot containing mRNAs from different tissues. The PCR-generated probe from the 3′ end of *MRP9* (Fig. 1*B*) was used for this analysis. As shown in Fig. 3*A*, a specific band of 4.5 kb in size is detected in testis. In contrast, a small band ≈1.3 kb in size was detected in brain and ovary (Fig. 3 *A* and *B*), suggesting that different variants of the *MRP9* transcript are expressed in different tissues.

Full-Length cDNA Cloning of *MRP9*. To isolate the 4.5-kb cDNA for *MRP9* we used conventional cDNA library screening as well as the 5′ and 3′ RACE-PCR method and isolated a clone of 4.5 kb in size from testis cDNA. The *MRP9* gene has 26 exons. Analysis of the complete nucleotide sequence of the cDNA reveals that it has an ORF of 930 aa and is made up of 20 exons. It lacks membrane-spanning regions 3, 4, 11, and 12 and the second nucleotide-binding domain normally present in a typical ABC C-type transporter (Fig. 1*C*).

A recent report by Tammur *et al.* (14) concluded that the *MRP9* gene is transcribed as a 5-kb transcript that encodes a 1,359-aa ORF that is expressed in testis, prostate, and ovary. As shown in Fig. 1*A*, the *MRP9* gene described by Tammur *et al.* has 29 exons (GenBank accession no. AY040220). However, the cDNA we isolated from testis has an ORF of only 930 aa. The major differences between the Tammur *et al.* sequence and our cDNA sequence are we do not detect exons 5, 16, and 26, and also we do detect an extra 30-bp sequence at the 5′ end of exon 22 (Fig. 1*B*). As a result, a stop codon TAG is present in our cDNA sequence, producing an ORF encoding a protein containing 930 aa. To verify this observation we PCR-cloned this region of the cDNA (Fig. 1*B*) by using the primer pair T396 and T399 from testis and normal breast cDNA and sequenced nine clones. Every clone contained the 30-bp extra sequence at the 5′ end of exon 22. To determine whether the variant, which does not contain the extra 30-bp sequence, can be detected in various tissues, we used a sensitive PCR-based analysis. We designed 5′ primers specific for each variant (T419 for the variant that contains the 30-bp extra sequence and T418 for the possible variant that does not contain the extra 30 bp; Fig. 1*D*). We used the same 3′ primer T399 for PCR amplification by using

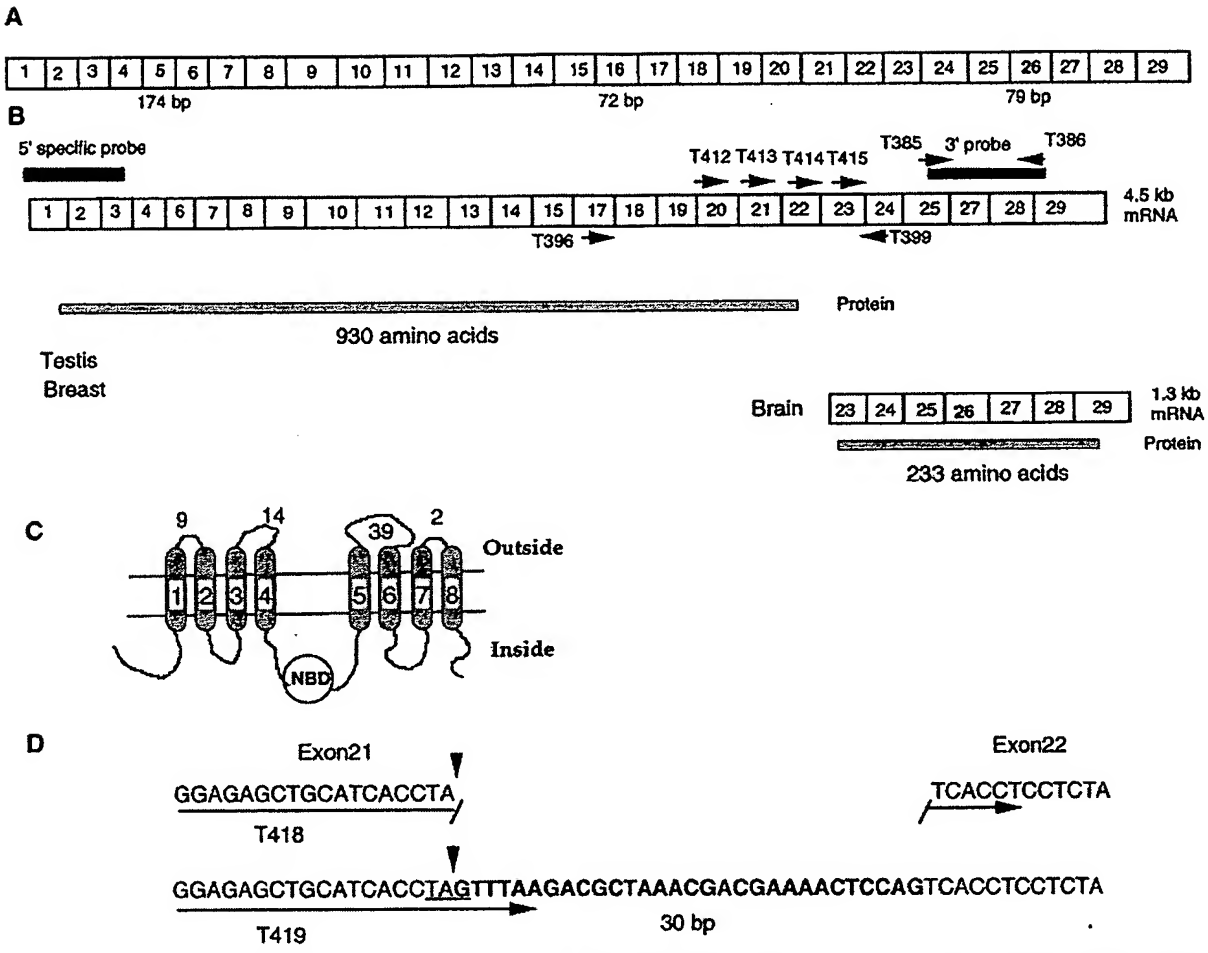

Fig. 1. Schematic of the *MRP9* cDNA and its variants. (*A*) Schematics of *MRP9* cDNA as described by Tammur *et al.* (14) (*B*) Variants of *MRP9* transcript and predicted ORFs. The 4.5-kb transcript has 26 exons, and the ORF starts at exon 1 and ends at exon 21. The 1.8-kb transcript has seven exons and has the ORF of 233 aa. The name and location of the PCR primers used are shown by arrows, and the location of the probes is shown by black rectangles. (*C*) Schematics showing the probable topology of the *MRP9*-translated protein. Eight possible membrane-spanning regions are numbered, and the number of amino acids exposed to the outside of the cells is mentioned. (*D*) Design and sequence of the PCR primer used in Fig. 4.

PCR-ready cDNAs from testis and breast. As shown in Fig. 4*A* (lanes 2 and 4), a specific 300-bp PCR product is detected only when primers T419 and T399 were used. No detectable PCR product was observed when primers T418 and T399 were used. This result shows that in both testis and breast the expressed *MRP9* transcript contains the extra 30-bp sequence at the 5′ end of the exon 22. Because the cDNAs used in this experiment are generated from pooled tissues from more than nine individuals, the presence of the extra 30-bp sequence represents a common splicing event and is not a rare event. In addition, there is deletion of 58, and 24 aa at positions 218 (exon 5) and 679 (exon 16), respectively, as compared with the Tammur *et al.* sequence (Fig. 1*A*). The 58-aa deletion at position 218 causes deletion of the third and fourth membrane-spanning regions normally present in a typical ABCC family transporter.

Analysis of the *MRP9* Transcript in Brain. The dot blot and rapid-scan PCR analysis shown in Fig. 2 indicates that *MRP9* is highly expressed in brain. The northern analysis in Fig. 3*A* shows that the transcript size of *MRP9* in brain is ≈1.3 kb, which is much smaller than the RNA detected in testis and breast cancer. To analyze the 1.3-kb transcript in brain, we used RACE-PCR with the T385 primer for 3′ RACE and the T386 primer for 5′ RACE (Fig. 1*B*). Marathon-Ready cDNA from brain was used as a template. The 5′-RACE reaction gave a DNA fragment of 850 bp, and the product from the 3′ RACE was ≈1.1 kb in size. Both the 5′- and 3′-RACE products were subsequently cloned in a TA cloning vector and sequenced. Results from the 3′-RACE analysis indicate that the sequence of the 3′ end of the 1.3-kb transcript in brain is exactly the same as the 3′ end of the 4.5-kb clone that we isolated from testis. All clones from brain generated by 5′ RACE started within exon 23, which indicates that the 1.3-kb transcript originates within exon 23. In addition, nine independent clones generated from brain RNA contain 79-bp exon 26 (Fig. 1*B*). The cDNA, which contains exon 26, has an ORF of 234 aa and encodes a nucleotide-binding domain that is missing in the protein encoded by the 4.5-kb variant of *MRP9*. To confirm the RACE-PCR analysis and rule out the possibility that we did not detect the 5′ end of the brain-specific transcript because of a limitation of the RACE reaction on a GC-rich template, we performed a PCR analysis on brain and testis cDNA by using several 5′ primers (T412, T413, T414, and T415, Fig. 4*A*) and T386 as 3′ primer (Fig. 1*B*). As shown in Fig. 4*B*, the expected sized PCR product was obtained with all four 5′

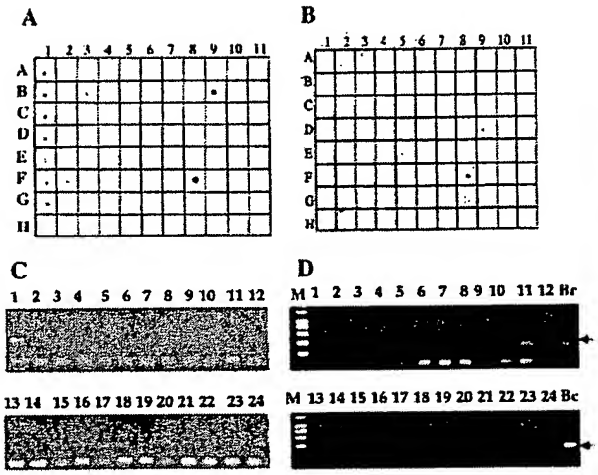

Fig. 2. Tissue distribution of *MRP9* mRNA expression. (*A*) RNA hybridization of a multiple-tissue dot blot containing mRNA from 50 normal human cell types or tissues using a cDNA probe from the 3′ end of the *MRP9* transcript. Signal is detected in testis (F8), pancreas (B9), and different parts of brain (A1, whole brain; B1, cerebral cortex; C1, frontal lobe; D1 parietal lobe; E1 occipital lobe; F1, temporal lobe; G1, paracentral gyrus cerebral cortex; A2, left cerebellum; B2, right cerebellum; D2 amygdala; and F2, hippocampus). There is a weak signal observed in the liver (A9), prostate (E8), and placenta (B8). (*B*) RNA hybridization of the same blot used in *A* with a 5′-specific probe. Specific signal is detected only in testis (F8). (*C*) PCR using 3′-specific primers on cDNA from 24 different human tissues (rapid-scan panel, OriGene); the expected size of the *MRP9* PCR product is 400 bp. Lanes: 1, brain; 2, heart; 3, kidney; 4, spleen; 5, liver; 6, colon; 7, lung; 8, small intestine; 9, muscle; 10, stomach; 11, testis; 12, placenta; 13, salivary gland; 14, thyroid gland; 15, adrenal gland; 16, pancreas; 17, ovary; 18, uterus; 19, prostate; 20, skin; 21, peripheral blood leukocytes; 22, bone marrow; 23, fetal brain; and 24, fetal liver. (*D*) PCR using 5′-specific primer pair on cDNA from 24 different human tissues. The expected size of the *MRP9* PCR product is 400 bp (shown by an arrow). The PCR product is detected in testis (lane 11), normal breast (lane Br), and breast cancer cell lines (lane Bc).

primers with testis cDNA, whereas with brain cDNA, only the T415 primer (which is within exon 23) gave a PCR product of 800 bp in size. Consistent with our RACE-PCR analysis, the major PCR product for brain cDNA with the T415 and T386 primer pair contains 79-bp exon 26. There is a very weak band of ≈700 bp in size, which probably accounts for the transcript without exon 26 (Fig. 4*B*, lane 4).

Long Transcript of *MRP9* Is Expressed Specifically in Testis and Breast. To determine whether the long form of *MRP9* is expressed specifically in certain tissues, we performed a multitissue dot blot

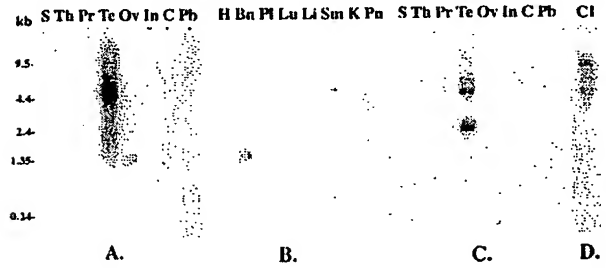

Fig. 3. Northern blot analysis showing expression and transcript sizes of *MRP9* in different normal tissues. Radiolabeled DNA probes from the 3′ (for *A* and *B*) and 5′ (*C* and *D*) ends of the *MRP9* cDNA used for hybridization. S, spleen; Th, thymus; Pr, prostate; Te, testis; Ov, ovary; In, small intestine; C, colon; Pb, peripheral blood leukocyte; H, heart; Bn, brain; Pl, placenta; Lu, lung; Li, liver; Sm, skeletal muscle; K, kidney; Pn, pancreas; and Cl, CRL1500.

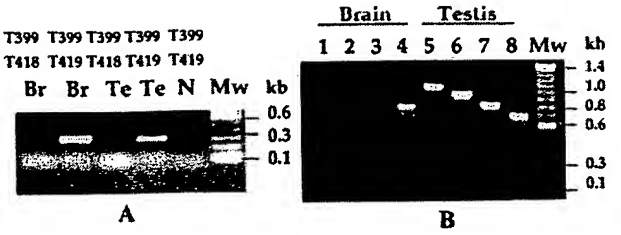

Fig. 4. PCR analysis of the *MRP9* variant in different tissues. (*A*) RT-PCR analysis of testis and breast RNA using either the T416/T399 or T417/T399 primer pair. Lanes 1 (T417/T399) and 2 (T416/T399), breast; lanes 3 (T417/T399) and 4 (T416/T399), testis. Lane 5 is negative control. MW, molecular weight standard. (*B*) RT-PCR analysis of brain and testis RNA with T412, T413, T414, and T415 as the 5′ primer and T386 as the 3′ primer. Lanes 1–4 are for primers T412, T413, T414, and T415, respectively, for brain; lanes 5–8 are for primers T412, T413, T414, and T415, respectively, for testis.

and rapid-scan analysis with a 5′-specific probe and a 5′-specific primer pair, respectively. As shown in Fig. 2*B*, among the 76 different samples of normal and fetal tissues tested in the dot blot, *MRP9* was detected only in testis. In the rapid-scan analysis shown in Fig. 2*D*, a specific band of 400 bp is detected in testis (lane 11), normal breast (lane Br), and breast cancer cDNA prepared from a pool of four breast cancer cell lines (lane Bc) but not in 23 other tissues tested including heart, brain, and lung. Subsequent analysis with breast cancer cell line RNA showed expression of *MRP9* in CRL1500 cells but not in three other breast cancer cell lines examined. When the 5′-specific probe was used in the multitissue Northern blot, a band of 4.5 kb in size was detected in testis and the breast cancer cell line CRL1500 (Fig. 3*C*). There is also a 2.4-kb band in testis, which could be a splice variant of the gene. No band was detected in any other tissues tested, which include ovary and brain (data not shown).

Expression of *MRP9* in Breast Cancer. To investigate whether *MRP9* is expressed in different samples of normal breast and primary breast cancers, an RT-PCR analysis was carried out by using a human breast cancer rapid-scan panel, which contains cDNAs from 12 different normal breast and breast cancer specimens. As shown in Fig. 5, using the PCR primer T385 and T386, the expected 400-bp PCR product was detected in 9 of 12 breast cancer samples. The signal was not detected in normal breast, although it was detected in one sample of normal breast RNA obtained from CLONTECH (Fig. 5).

MRP9 mRNA Is Expressed in the Epithelial Cells of Breast Cancer. To determine whether the longer 4.5-kb variant of *MRP9* mRNA is expressed in epithelial cells of a breast cancer specimen from patients, we used *in situ* hybridization with a biotin-labeled 5′-specific *MRP9* cDNA (nucleotides 1–604). *MRP9* mRNA is expressed only in the epithelial cells (Fig. 6). A representative example of a strong signal over the breast cancer cells demonstrates no detectable signal in cells of the stromal compartment of the tissue.

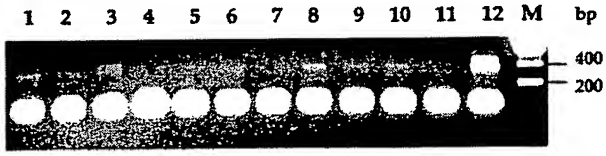

Fig. 5. Rapid-scan PCR analysis using a 3′-specific primer pair on cDNAs from 12 different breast cancer specimens (lanes 1–12). M, molecular weight marker.

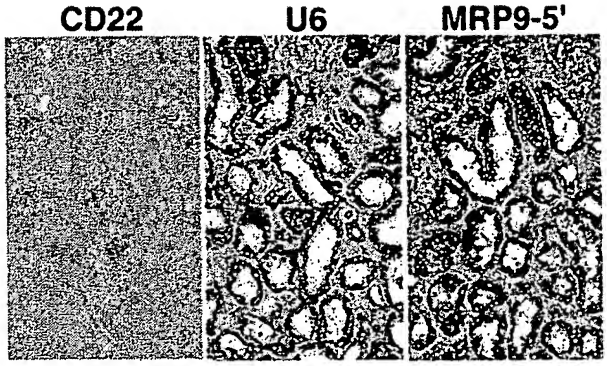

Fig. 6. *In situ* localization of *MRP9* mRNA. Shown are breast cancer tissue sections stained with CD22 and U6 probe used as a negative control (CD22) and positive control (U6), respectively. A serial section of the same cancer tissue stained with a 5'-specific *MRP9* probe (*MRP9*-5') also is shown. Note the strong signal in the tumor cells.

***In Vitro* Transcription and Translation of the *MRP9* cDNA.** The *MRP9* cDNA has a predicted ORF of 930 aa with a calculated molecular mass of 95 kDa. To determine the size of the protein encoded by the *MRP9* cDNA, *in vitro* transcription and translation was performed by using the rabbit reticulocyte lysate system. SDS/PAGE analysis and fluorography of the translated product showed a doublet of ≈100 kDa in size (Fig. 7*A*), perhaps because of a different amount of glycosylation. The size of the protein products agrees with the predicted ORF of the cDNA.

The *MRP9* Transcript Encodes a 100-kDa Membrane Protein. To identify the protein expressed by the *MRP9* gene, we developed polyclonal antibodies in rabbits against a synthetic peptide (amino acids 15–28) of *MRP9*. By using a purified IgG fraction of the antisera, a doublet band at a molecular mass of ≈100 kDa, was detected in testis but not in brain, heart, liver, kidney, or prostate samples (Fig. 7*B*). A similar band was detected in the total membrane fraction prepared from the CRL1500 breast cancer cell line (Fig. 7*C*, lane Me). We did not detect any specific bands with IgG prepared from the preimmune serum (data not shown).

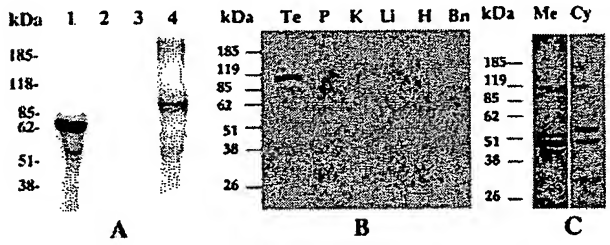

Fig. 7. Analysis of the protein product encoded by the 4.5-kb variant of *MRP9*. (*A*) Analysis of the *in vitro* translated products of *MRP9* cDNA. The 4.5-kb variant of *MRP9* cDNA was transcribed *in vitro* with T7 RNA polymerase and couple-translated with rabbit reticulocyte lysate in the presence of [^{35}S]methionine. The translated products were analyzed by SDS/PAGE and fluorography. Lane 1, luciferase cDNA as positive control; lane 2, no DNA; lane 3, *MRP9* cDNA in antisense orientation; and lane 4, *MRP9* cDNA in sense orientation. (*B* and *C*) Western blot analysis of anti-*MRP9* peptide antisera. A specific protein with a molecular mass of ≈100 kDa is detected by anti-*MRP9* IgG in testis (Te) tissue extract and in membrane (Me) fraction of the CRL1500 extract (C). The tissue extract from brain (Bn), heart (H), liver (Li), kidney (K), prostate (P), and the cytoplasmic (Cy) fraction of CRL1500 showed no detectable signal.

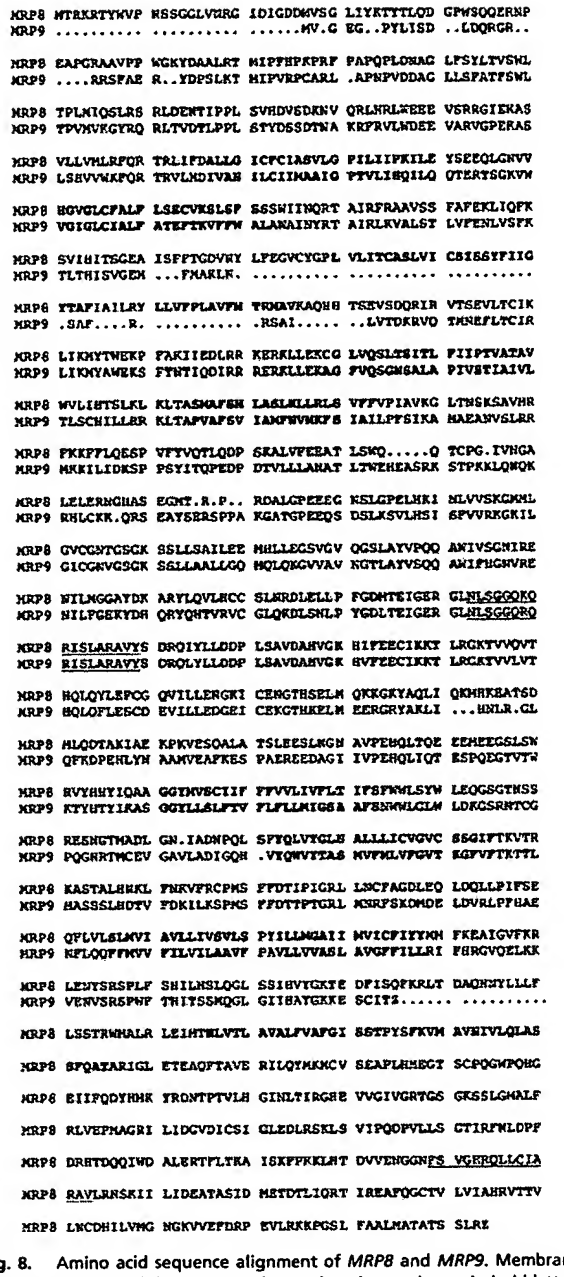

Fig. 8. Amino acid sequence alignment of *MRP8* and *MRP9*. Membrane-spanning regions of the transmembrane domain are shown in bold letters, and the conserved ABC signature motifs for both *MRP8* and *MRP9* are underlined.

Discussion

We have used a functional genomic approach and bioinformatics tools to identify *MRP9* (ABCC12), a member of the ABC transporter superfamily. Our experimental data show that the *MRP9* transcript is expressed as different variants in different tissues. The larger 4.5-kb transcript is highly expressed in breast cancer and testis and weakly expressed in normal breast. It encodes a protein of ≈100 kDa molecular mass. The smaller 1.3-kb transcript is expressed in brain, skeletal muscle, and ovary. The smaller transcript has an ORF of 234 aa.

MRP9 Is a Unique Member of the ABCC Family. The multidrug resistance (MDR)/ABC superfamily of membrane transporters is one of the largest protein families and is involved in energy-dependent transport of a variety of substrates across the membrane including drugs used to treat cancer (15–17). In humans this superfamily is divided further into seven subfamilies (ABC-A to -G) based primarily on sequence similarity. Most ABC proteins from eukaryotes encode full transporters, consisting of two ATP-binding domains and 12 membrane-spanning regions or half transporters, which are presumed to dimerize (16, 18). We described earlier that the sequence of MRP8, which is related closely to MRP5, belongs to the ABCC subfamily. MRP8, similar to other members of the subfamily, is a full transporter with two nucleotide-binding and 12 transmembrane-spanning regions. The MRP9 sequence, similar to that of MRP8, is related closely to MRP5 (19), with an overall 44% identity and 55% sequence similarity at the protein level. Between MRP8 and MRP9, the overall sequence identity and similarity is 47 and 56%, respectively. One major difference between MRP8 and MRP9 is that MRP9 has only one ATP-binding domain but two transmembrane domains each with four membrane-spanning regions. A few so-called half-transporters with one ATP-binding domain and six membrane-spanning regions have been reported and characterized (20–22). The two half-transporter molecules normally are transcribed separately, translated, and then probably assembled together to generate a full transporter. However, in the case of MRP9, a premature stop codon truncates the protein and generates an unusual protein without the second ATP-binding domain and containing only four membrane-spanning regions in the carboxyl half of the protein. In addition, the 58-aa deletion within the amino-terminal half of MRP9 causes deletion of the third and fourth membrane-spanning regions of the molecule (Fig. 8). The importance of the loss of four membrane-spanning regions and one nucleotide-binding domain is unknown because MRP9 lies adjacent to MRP8, and it probably arose by gene duplication and then underwent further mutational changes to carry a new and different function.

The Smaller 1.3-kb MRP9 Transcript Is Caused by an Alternate Transcription Start Site. Different sized mRNAs of the ABCC family members often are observed during Northern analysis. In most cases, different sized mRNAs arise because of alternate splicing of the major transcript. In the case of *MRP9*, the Northern analysis using a 3′ probe (Fig. 3*A*) shows that in brain and ovary the transcript is ≈1.3 kb, whereas the transcript detected in testis is ≈4.5 kb. When the 5′-specific probe was used in Northern analysis (Fig. 3*B*), only the 4.5-kb transcript of testis was detected, indicating that both the 5′ and 3′ probes are recognizing the same transcript. The 5′ probe does not recognize the 1.3-kb transcript of *MRP9* from either ovary or brain. RACE-PCR cloning of the full-length 1.3-kb variant of *MRP9* from brain and RT-PCR analysis also suggest that the 1.3-kb variant is transcribed independently and not caused by an alternate splicing event. This 1.3-kb transcript has an ORF of 234 aa and encodes one of the ATP-binding domains of the transporter molecule. It will be interesting to determine whether the protein encoded by this transcript is expressed in the tissue by generating specific antibody against the ORF. Also, its biological function is unknown.

The MRP9 Variant Is a Potential Candidate for Immunotherapy. Both RT-PCR analysis by a 5′-specific primer pair and the Northern blot and *in situ* analysis using a 5′-specific probe indicate that the larger 4.5-kb *MRP9* transcript is expressed selectively in breast cancer, normal breast, and testis. However, the 1.3-kb transcript is very nonspecific. Recently Yabuuchi *et al.* (23) reported multiple splice variants of *MRP9* (ABCC12) in various adult tissues including brain, lung, liver, kidney, pancreas, and colon. The transcripts were detected by PCR with primers from the 3′ end of the gene. Our results indicate that the 1.3-kb variant of *MRP9* is expressed in several adult tissues and likely represents the transcript that Yabuuchi *et al.* detected. The longer 4.5-kb transcript is expressed specifically in breast cancer, normal breast, and testis. Our *in situ* RNA analysis (unpublished data) confirms the RT-PCR results and confirms that many cancer specimens are positive for *MRP9* expression. Because *MRP9* is a membrane protein and it has very restricted expression in essential tissues, it is a potential target for targeted therapy with antibodies, antibody conjugates, and immunotoxins.

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